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ON THE ORIGIN AND NATURE OF THE MIDDLE LAMELLA.

CHARLES E. ALLEN.

THERE has long been recognized in the cell walls of plant tissues, and particularly in the thickened walls of bast and xylem elements in woody plants, a central layer or plate, sharply distinguishable by its optical and staining properties from other regions of the wall. To account for the origin of this layer, apparently homogeneous and equally closely related to each of the cells between which it occurs, various theories have been advanced, and the names "primary cell wall," "intercellular substance," and "middle lamella" indicate the diversity of opinion that has prevailed as to its real nature.

The theories of the origin of this "middle lamella," adopting as most convenient its commonest designation, may (1), generally speaking, be grouped under three heads: (1) those which hold it to have been originally a common matrix in which the cells were imbedded; (2) the theory that the middle lamella is the original cell wall, laid down in common by the two daughter cells in the process of cell division, and remaining distinct from the secondary thickening layers deposited upon its either face; and (3) the view that it is a substance excreted by the daughter cells into a space left between them after their formation, or into a space formed by their rounding up and drawing apart.

The first theory, that the middle lamella is an original common matrix of the cells, was shown to be groundless as soon as

any satisfactory investigation of the process of cell division could be made.

The notion of the middle lamella as the primary cell wall until very recently was the most prevalent, and is the one still laid down in most text-books of botany. Vines (**15**) thus states this view:

> In the development of a tissue, whether by free cell formation or by cell division, septa are formed, that is, walls which are common to contiguous cells; these are very thin at first, and appear under the highest magnifying power as a simple plate. As the walls increase in thickness and acquire a more or less distinctly stratified structure, as seen in transverse section, the network of primary septa stands out from the thickening layers proper to each cell. The primary septum between any two cells is now distinguished as the *middle lamella* (sometimes also termed *intercellular substance*); it attains a considerable bulk at points where several septa meet at an angle.

This theory was early stated by Strasburger, and until lately he has adhered to it in unmodified form. In the first edition of the *Botanisches Practicum* (**10**), on p. 78, he describes a method of demonstrating the presence of this median layer in the endosperm of the seed of *Ornithogalum umbellatum* by treatment with sulfuric acid, the delicate network of the middle lamella being the last part of the cell walls to dissolve. This network, he says, corresponds to the original walls present before the process of thickening began. In another passage, on page 82, speaking of the tracheids of *Pinus silvestris*, he uses the term "primary wall," which, however, is not the same as the first wall laid down in cell division—the middle lamella—but includes the latter as a very delicate layer (*Theilplatte*). The middle lamella differs from the rest of the primary wall in being "cutinized." To the original partition laid down in the process of cell division, Strasburger (**9**) had in 1875 given the name "cell plate."

Treub (**14**), in 1878, studying the living cells of the proembryo of *Orchis latifolia* and the ovules of *Epipactis palustris*, found, after its formation, a splitting of the cell plate, and the deposition of a cell wall between the two layers so formed. This is, I believe, the first suggestion that the median layer of the cell wall is not the plate first laid down in the process of cell

division, but is a substance secreted into a space between the daughter cells.

The history of the cell plate theory has been carefully summarized by Timberlake (**13**), and reference may be made to his paper for the literature of the subject. The results of recent investigations go to show the essential correctness of Treub's view. In 1898, Strasburger (**12**) announced that the cell plate splits to form a plasma membrane for each daughter cell. During the splitting process, the rod-shaped elements visible in the cell plate, which represent the material furnished to the plate by the spindle fibers, are pulled out so as to become extremely thin in their middle portion. A middle layer then appears between the halves of the original layer. Whether or not the thread-like portions of the rods persist, either as part of this new layer or as protoplasmic connections between the plasma membranes of the daughter cells, he did not determine. So the question was left open whether the substance of the wall arises entirely by secretion into the space formed by the splitting of the cell plate, or whether a portion of the original cell plate takes part in its formation; but Strasburger has wholly abandoned his old notion of the identity of the middle lamella with the original cell plate.

Timberlake (**13**) finds in the cell divisions of the root tip of *Allium Cepa*, before the appearance of the cell plate elements, an accumulation of an orange-staining substance in the equatorial zone of the spindle. From the staining reactions of this substance, he concludes that it is some form of carbohydrate, probably destined for the building of the wall between the daughter cells. But, while in general the material of this zone stains like the cell wall, it is not stained, as is the wall, by either ruthenium red or iron hæmatoxylin. The cell plate is formed in the midst of this zone by the fusing together of equatorial thickenings of the spindle fibers. The carbohydrate material disappears with the formation of the cell plate, but very soon after the splitting of the plate the young cell wall (middle lamella) appears in the cleft. The cell plate begins to split in its oldest part, that is, in the central portion of the spindle, so

that a cell wall may be visible here before the cell plate by peripheral growth has reached the mother cell wall.

Mangin (**3, 4, 5, 6, 7, 8**), in a series of contributions which appeared from 1888 to 1893, investigated particularly the chemical nature of the substance in question. As this side of the subject and Mangin's very interesting conclusions regarding it have been little discussed, a somewhat full résumé of his results may here be given.

Dippel, quoted by Mangin (**3**), had already announced that the new partition deposited by the protoplasm has none of the properties of cellulose. Frémy found in the tissues of fruits and roots a substance called by him pectose, which he was unable to separate from cellulose, and from which are produced the pectic compounds found in the walls of fruits. Maudet found in the pith of certain trees pectose and calcium pectate, forming a cement which holds the cells together. Mangin (**3**) found plant cell walls to be generally formed by the association of two substances, cellulose and one which he provisionally called pectose. In a large variety of adult tissues he found pectose, in a pure state, forming the middle lamella or intercellular substance, and associated with cellulose in the other layers of the cell wall. Dippel's conclusion, that the middle lamella is free from cellulose, was thus confirmed. Some tissues were found to be composed wholly of pectose. It plays the principal rôle in what had been called cellulose fermentation, and Mangin considered chemical modifications of cell walls, as lignification and cutinization, to be due to transformations of pectose, of which cellulose is incapable. At this time (1888) he repeatedly speaks of the pectose layer as the first membrane formed in cell division, the "fundamental layer of the cellular membrane." This seems to indicate an acceptance of the view then held by Strasburger as to the identity of the middle lamella with the cell plate.

But in 1890, Mangin (**5**) suggested the appropriateness of restoring to this layer the name "intercellular substance," as expressing better than that of "middle lamella" its origin and

mode of formation. He found it to consist, not of pectose proper, but of a cement of insoluble pectates. To demonstrate the presence of pectic acid, tissues are macerated in a mixture of alcohol and hydrochloric acid, and then treated with a weakly alkaline solution. The tissues dissociate into their constituent cells and fibers, the intercellular substance passing into solution. The solution is shown by analysis to contain pectic acid, which appropriate staining now shows to be absent from the cell walls. In this process, the insoluble pectates are first changed into pectic acid, which, in the alkaline solution, forms a soluble alkaline salt. If sections first treated with acid alcohol are stained with a pectic stain, as phenosafranin or methylene blue, the pectic acid present stains more deeply than the pectic compounds associated with cellulose in the wall layers lying between the intercellular substance and the cell cavity. This indicates that it is not pectic acid and its derivatives, but some of the neutral pectic substances, as pectin or pectose, which are in combination with cellulose in the later deposited layers.

The intercellular substance thus deeply stained forms a thin layer on the whole surface of contact of adult cells; where the cells draw apart, it produces a thick cushion; when the cells separate so as to form intercellular spaces, these spaces are bounded by a pectic layer. This frame of pectic acid is sometimes thickened irregularly, so as to form knobs, points, and sculptures of various forms ornamenting the frame itself or projecting into the intercellular spaces. Sometimes the spaces are partially or completely filled with a jelly-like mass, a soluble transformation product of pectic acid. In the meristem the intercellular layer is not disclosed by staining, but the chemical reactions of cellulose and pectic compounds are given by the cell membranes. That a thin layer of intercellular substance is present is shown by the dissociation of the cells as in older tissues after treatment with acid alcohol and an alkaline solution. Mangin explains the variations in thickness and form of the intercellular cement by its partial transformation in the course of the development of the tissues into soluble pectates; this

makes possible the splitting of the membrane, the formation of intercellular spaces, and an exudation of material to form the knobs, points, and other structures already referred to.

A further proof of the presence of pectic compounds in the wall is furnished by treating tissues with a cellulose solvent; the general outlines of the cells remain, the middle lamella, and often a large part, or in some cases all, of the other wall layers preserving their form. The framework thus left takes up pectic acid stains and is indifferent to cellulose stains. The best distinctive stain for pectic substances is ruthenium red, the ammoniacal sesquichlorid of ruthenium. Our knowledge of the value of this subtance is due to Mangin (**8**), who first described its remarkable properties as a staining reagent for vegetable tissues.

Mangin's attention, it is seen, has been directed to the chemical composition and transformations of the "intercellular substance," and he has not connected his valuable results obtained in that field with a study of its origin. It was in part to supplement his work in this important regard that the present investigation was undertaken.

Strasburger (**12**) and Dippel (**2**) accept Mangin's conclusions as to the widespread presence and the importance of pectic substances in plant tissues, especially in the middle lamella and the thin layer immediately surrounding the intercellular spaces. Dippel's account of the history of the middle lamella in the wood and bast of higher plants is briefly as follows (pp. 570 ff.).

The radial walls of dividing cambium cells are separated from one another by a lax, weakly refractive *Zwischenmasse* or *Zwischensubstanz*. This substance decreases in amount as we pass outward from the cambium layer into the differentiated wood and bast tissues, until it finally disappears, excepting at the angles formed by the junction of three or four cells. Remains of the cambium mother cell walls may occasionally be recognized in the *Zwischensubstanz*, which Dippel believes to be at least partially produced by their disorganization. Chemically, the *Zwischensubstanz* may consist of a union of pectose and callose, which is soluble under the conditions of cell wall development,

and so, for the most part, is absorbed into the cambial daughter walls. The cambial cell walls (p. 575), that is the cell walls of the cambium mother[1] and daughter cells, are composed of pectic acid, which, at least after the transformation of these walls into an "intercellular substance," exists largely in the form of calcium pectate. As the cambial daughter cells are transformed into bast and wood tissue, the primary cell walls, consisting of pectose and cellulose, are deposited next to the now apparently simple cambial walls. These latter now undergo a transformation, forming an "intercellular substance," or *Kittsubstanz*, which, through a loss of water and the extension in length of the radial walls, becomes so thin as to be in most cases invisible except after special treatment. It is, besides, fused into apparently intimate connection with the primary cell walls. This combination of the intercellular substance and the primary cell walls forms the middle lamella of mature tissues, against which in the process of development secondary thickenings are deposited. The continued existence of the "intercellular substance" as a middle plate (*Theilplatte*) of the middle lamella Dippel demonstrates by the action of the wall layers upon polarized light, as well as by treatment with various macerating and staining reagents, notably ruthenium red.

Dippel's peculiar view, developed in connection with his studies on the algae, that cell division is accompanied by the formation of a new cell wall entirely enclosing each daughter cell, has doubtless influenced his account of the development of the middle lamella in the higher plants. For if this were the case in the predominantly radial growth of cambium cells, the result would be to leave, between the radial walls of the newly-formed daughter cells, the old mother cell walls, which, if they became disorganized, would form a *Zwischensubstanz* such as Dippel describes. The tenability of this view of the origin of the *Zwischensubstanz* will be discussed hereafter.

[1] Here there seems to be a contradiction, for Dippel has already spoken (p. 575) of the cambium mother cell walls as being disorganized to form the *Zwischensubstanz* of the daughter cell walls.

MATERIAL AND METHODS.

Woody tissues of the following species were studied: *Pteris aquilina* L., *Pinus silvestris* L., *Nerium Oleander* L., *Rosa* sp., *Tilia Americana* L., and *Ilex opaca* Ait. Of these, Mangin had investigated tissues of Pinus and Ilex.

Free-hand sections were cut, usually both transverse and longitudinal. In many cases, before staining, they were treated for twenty-four hours, as suggested by Mangin (**5**), with a mixture of one part of concentrated hydrochloric acid and four or five of absolute alcohol, then washed in distilled water. The stains characteristic of pectic acid, especially ruthenium red, are taken up, as a rule, more freely after this treatment, and almost invariably the coloration is more clear-cut and distinctive.

For staining, Grübler's preparations were used. Unless otherwise stated, they were in neutral saturated solution. When an alkaline solution was used, it was prepared by adding to the neutral solution about ½ per cent. of concentrated ammonia solution. Acidulated solutions were prepared by adding a like proportion of acetic acid. In some cases where saturated solutions, *e. g.*, of methylene blue and methyl violet B, were too strong for good results, they were diluted to such a strength that a few seconds' exposure gave a differential stain. Aniline-water-safranin was prepared as directed by Zimmermann (**16**). An aqueous solution of ruthenium red of a strength of about one five-thousandth was used. An increase in strength above this did not seem to make any noticeable difference in the depth of the stain or in the time of exposure required. This substance is reduced by the action of light in the presence of water, hence the solution must be kept in the dark. It was tried in neutral, alkaline, and acidulated solutions, the alkaline and acidulated solutions being prepared as described for the other coloring matters, substituting hydrochloric for acetic acid. In all cases, the alkaline solution was found to give the deepest and most distinctive colorings, and it is this preparation of ruthenium red that is referred to hereafter. But a few minutes' exposure to the stain is required.

In general, sections after staining were mounted in water and studied immediately. Ruthenium red preparations can be dehydrated by absolute alcohol, passed through clove oil, and mounted in Canada balsam without affecting the stain. But the color gradually fades out from sections so treated, some tissues, notably the xylem and bast, losing their color almost entirely within a few weeks, while others, as the parenchyma and pith, retain it much longer. Sections stained with ruthenium red and mounted in glycerin jelly lose their color almost entirely in the course of a few hours.

Mangin (**4, 6, 7, 8**) has classified the coloring matters used in staining plant tissues, and has described their reactions upon the various substances found in cell walls. Of the great number of stains named by him, the following were employed in this investigation:

Orseille, which colors cellulose in neutral or slightly acidulated solution, but does not affect callose.

Aniline-water-safranin (phenosafranin), methylene blue, Bismarck brown (Vesuvian brown), fuchsin, and methyl violet B (violet de Paris), which do not color callose or cellulose, but do color pectic acid in neutral or acidulated solution. They also color equally well nitrogenous substances (lignin, cutin, etc.), but these may be distinguished by their retention of the color after treatment with alcohol, glycerin, or acids, which treatment decolorizes pectic acid. According to Strasburger (**11**), safranin colors the protoplasmic contents of the cell, the lignified wall, cork, and cutinized membranes cherry-red, the pectic substances orange-yellow. Methylene blue colors the protoplasmic contents and lignified walls blue, the pectic substances violet-blue.

Acid brown, nigrosin, and ponceau, which do not color pectic compounds, but strongly color nitrogenous substances. By mixing one of this series with one of the preceding, Mangin obtained distinctive double stains.

Ruthenium red, which Mangin found the most satisfactory distinctive stain for pectic compounds. It differs from the other pectic stains, as safranin and methylene blue, by the fact that

pectic compounds stained by it are not decolorized by glycerin or alcohol. Ruthenium red, he finds, also stains gums and mucilages formed by the decomposition of pectic substances, but does not affect decomposition products of cellulose or callose. Lignified tissues, not stained by ruthenium red when fresh or preserved in alcohol, take it up after treatment with alkalies or Javelle water; but their affinity for ruthenium red is always less than for certain basic organic stains, such as methylene blue, so that by combining its action with that of one of the latter class fine double colorations may be obtained. Cutinized membranes, according to Mangin, are stained in many cases, but not the cuticle. Ruthenium red also stains in varying degrees the protoplasmic cell contents.

PTERIS AQUILINA.

The rhizome was studied in cross section. In unstained sections the middle lamella of the sclerenchyma walls is plainly distinguishable from the inner layers by its greater density and refractive power. It appears as a dark yellowish-brown layer constituting a considerable proportion of the total thickness of the wall, enlarged at the angles where three or four cells abut into a triangular or quadrangular form, often enclosing at such places a similarly shaped intercellular space.

In the walls of the stone cells, while the absolute thickness of the middle lamella is approximately the same as in the sclerenchyma, its thickness relatively to that of the whole wall is of course considerably less. Otherwise its appearance is much the same as in the sclerenchyma. The canals appear as transverse lines seeming to pass quite through the wall, including the middle lamella.

On treatment with methylene blue, the sclerenchyma wall is colored green, except the middle lamella, which, if stained at all, yet appears yellowish-brown in contrast with the other layers. The walls of the fundamental parenchyma, as well as the protoplasmic cell contents, are stained blue. The parenchyma walls are apparently continuous with, and of the same thickness

as, the middle lamellae of the sclerenchyma. The green appearance of the sclerenchyma walls is probably not a differential coloring, but is due to a combination of their original yellowish-brown with the blue stain.

In sections treated with ruthenium red, the sclerenchyma and stone cell walls do not take up the stain to any appreciable extent. The walls of the fundamental parenchyma are clearly brought out, and are again plainly continuous with the middle lamellae of the sclerenchyma and stone cells. The indifference of the middle lamella in these thickened walls to methylene blue and ruthenium red, although this layer is directly continuous with the thin, readily-staining parenchyma wall, is in marked contrast to its character in the tissues of the spermatophytes studied; it may be due to a change in the chemical composition of the middle lamella which occurred simultaneously with the deposition of secondary thickening layers. The fundamental parenchyma walls show no evidence of secondary thickening. They are stained by ruthenium red deeply and quite uniformly, save that at the angles the wall is thicker, the triangular or quadrangular area so formed is less deeply stained, and contains apparently a less dense substance than that forming the remainder of the wall. This less deeply stained area corresponds to the thickened corners and the intercellular spaces of the sclerenchyma. The protoplasmic contents of the parenchyma cells are stained unevenly, the nuclei very deeply.

In the vascular bundles, the parenchyma walls are deeply and uniformly stained by ruthenium red. These walls appear continuous with the stained middle lamellae of the large vessels and sieve tubes. In the walls of the vessels the middle lamella is relatively thin; the rest of the wall is uncolored. The middle lamella of the sieve tubes occupies a larger proportion of the wall thickness, the interior unstained layers being very thin. At corners formed by three or four cells the middle lamella appears more dense and somewhat, though not greatly, enlarged.

The staining reaction of the middle lamella in these elements is in contrast to its conduct in the sclerenchyma and stone cells,

and agrees with results generally noted in tissues of spermatophytes.

PINUS SILVESTRIS.

Sections of young twigs and branches cut in the fall were used; these, therefore, were past the season of rapid cambial division, and the cambium cells were in a condition of comparative rest. Sections of old and seasoned pine wood were also used for comparison with the young tissues.

In cross sections through the cambium and neighboring tissues of young wood, treated with acid alcohol as above described, the cambium walls are stained throughout their thickness by methylene blue a deep and apparently uniform blue. The result is similar in sections not previously treated with acid alcohol.

Similar cross sections, both in the fresh condition and after treatment with acid alcohol, were stained with ruthenium red. This stain is also taken up freely by the cambium walls. The radial walls are noticeably thicker than the tangential. In the middle of the radial walls there is often a less deeply staining layer, recognizable as Dippel's *Zwischensubstanz*. The rest of the walls are deeply stained, except that in many of the older cambium cells, whose walls are thicker than those of the youngest cells present, there is, next the cell interior, a light line indicating a later deposited, unstained, or less deeply stained wall layer. The corners where radial and tangential walls join are especially deeply stained. In many cases, a continuation of the radial cambium wall into the middle lamella of the xylem and bast can easily be traced.

A difference in depth of stain between radial and tangential walls similar to that given by methylene blue is found in the longitudinal sections treated with acid alcohol and exposed for a few minutes to the action of Bismarck brown.

In cross sections treated with acid alcohol and stained one hour with fuchsin, the cambium walls are uncolored; the protoplasmic contents of some of the cambium cells are stained red. The difference between the affinity of these walls for Bismarck

brown and fuchsin is worthy of note; both these stains belong to the class in which a basic coloring matter is united to an acid; according to Mangin, all the stains of this class stain pectic compounds and do not color cellulose or callose.

In unstained cross sections through the xylem, the middle lamella is to be distinguished from the rest of the wall by its different refractive power, at some levels of focusing appearing brighter, at other levels darker, than adjacent layers. The same is the case in cross sections through old pine wood, except that here the middle lamella appears, if anything, more dense than in the young wood.

In cross sections through young wood treated with acid alcohol and methylene blue, the whole xylem is colored green. The middle lamella is distinguishable only by its greater density, as also in sections not previously treated with acid alcohol.

Xylem walls in cross sections treated with acid alcohol stain more deeply with ruthenium red than in sections not so treated. The middle lamella stains much more deeply than the inner layers, the boundary between the lightly and deeply stained portions being sharply marked. The difference in depth of stain is much more apparent in acid alcohol sections than in the others. The tori of the pits in acid alcohol sections are stained deep red; this occasionally occurs also in sections not treated with acid alcohol. In some cases in acid alcohol sections, the torus appears plainly to be a continuation of the middle lamella. In some cases also the middle lamella appears continuous with the interior layer of the pit cavity, which stains deeply. The middle lamella is more plainly differentiated in xylem of the present year's growth. In some preparations the oldest cells of the present year's growth (the spring wood) have the whole wall quite deeply stained. These facts may indicate that it is in the younger xylem walls that pectic substances exist in purest form. Where splitting of the walls occurs in preparing the section, it is sometimes on the line of demarcation between the middle lamella and the less stained layers; very often, however, the split is approximately through the center of the middle

lamella. Intercellular spaces occur in the xylem where three or four cells abut; they appear as triangular or quadrangular breaks in the uniformly stained middle lamella.

Comparing the results obtained from methylene blue and ruthenium red, we find that by both the whole xylem wall is stained, the middle lamella being differentiated by the red and not by the blue. Since methylene blue stains nitrogenous substances equally well with pectic acid, and does not stain cellulose or callose, while ruthenium red stains pectic derivatives and not nitrogenous substances, it appears that the secondary layers of the xylem, as well as the thin layer of the cambium wall which does not take up ruthenium red, contain a mixture of nitrogenous and pectic compounds. The use of orseille, a cellulose stain, gave no satisfactory results, showing that celulose, if present at all in these walls, is of minor importance. The staining power of the tori of the pits indicates that they represent in the main the same layer as the middle lamella; this does not, however, preclude the possibility of their also possessing a thin, subsequently deposited layer. The deeply stained layer lining the pits is an example, of which others will be mentioned later, of the possible deposition of a pectic layer very late in the history of the growth of the cell wall.

The relations of the middle lamella, tori, intercellular spaces, and thickening layers appear about the same in older pine wood stained with ruthenium red as in the young tissues already described.

In cross sections previously treated with acid alcohol and exposed for one hour to fuchsin, which belongs to the same class as methylene blue, the xylem walls are stained throughout. Other tissues, except the contents of the cambium and collenchyma cells, are unstained.

Cross sections of older pine wood were left in ponceau solution for three or four days. The xylem is quite uniformly stained vermillion, the middle lamella generally appearing darker than the inner layers of the wall, but the distinction of coloration is not strongly marked, and the difference may be wholly due to the difference in density.

The xylem walls of young wood are not stained after a half-hour's treatment with acidulated nigrosin solution. This fact, by itself, would mean the absence of nitrogenous substances; but, as against that furnished by methylene blue, and by ponceau, which belongs to the same class as nigrosin, such negative evidence is of little value.

In longitudinal sections of young tissues treated with acid alcohol and stained with ruthenium red, the tori of the pits are stained deep red. The appearance of the tori and of the red-staining bars between the pits, which bars are referred by Dippel (2) to the remains of the *Zwischensubstanz*, is well shown by him in his figure 397. In many cases, probably in partly developed pits, the stain is only a deep spot at the center. In some cases, an unstained center is surrounded by a stained ring. The pits are not so noticeable where they connect with the cells of the medullary rays, but where the ring appearance just described is found in older wood, the tori of pits so situated are completely stained.

Both the walls and the cell contents, especially the nuclei, of the medullary rays are deeply stained by methylene blue, both in cross sections previously treated with acid alcohol and in those not so treated. This is true of both young and old wood. Similar results are effected by ruthenium red. In cross sections of old wood stained with ruthenium red and then exposed for a few seconds to methylene blue, the red stain of the xylem is replaced by the blue, while, if the action of the blue has not been too long continued, the medullary rays remain red. The greater affinity of the xylem walls for the blue than for the red is due probably to their containing nitrogenous and pectic compounds, both of which take up methylene blue, while the former, unless specially treated, have no affinity for ruthenium red.

In cross sections treated for one half hour with acidulated nigrosin solution, the medullary rays and their cell-contents take up the stain freely.

In cross sections of older pine wood treated for three or four days with ponceau, the medullary rays are unstained.

The readiness with which the walls of the medullary rays take up methylene blue and ruthenium red, and also nigrosin, indicates the presence in them of both pectic and nitrogenous substances. Their preference for ruthenium red indicates a greater proportion of pectic constituents than in the xylem walls. But against this view is the negative evidence of the ponceau, though this, perhaps, is not of much weight.

The cells about the resin pits and their contents, if any, are always deeply stained by either methylene blue or ruthenium red. This indicates, according to Mangin, that the resin and other substances formed in the breaking down of these cells are wholly or chiefly decomposition products of pectic acid and its derivatives.

The staining of the bast is in general similar to that of the xylem. In both acid alcohol and fresh sections, the whole wall takes up more or less freely either methylene blue or ruthenium red, the middle lamella being much more deeply stained than the inner layers. The middle lamella, of course, is thicker relatively to the total thickness of the wall, but is of about the same absolute thickness as in the xylem.

The middle lamella of the collenchyma walls is generally stained more deeply than the other wall layers by methylene blue or ruthenium red, especially in sections treated with acid alcohol. Here, except at the corners, the unstained portion of the wall is very thin, and often the whole wall appears quite uniformly stained. The stain appears more diffuse than in the xylem, and the distinction between the more and less deeply stained layers is not so sharp. The middle lamella is enlarged at the angles of the cells, and sometimes encloses a triangular or quadrangular intercellular space.

The appearance of the collenchyma is very much the same in longitudinal as in cross sections. The collenchyma walls become red when treated for thirty minutes with acidulated orseille solution, indicating the presence of some cellulose, but no distinctive stain for different portions of the wall is observable.

The walls, except the outer ones, of the epidermal cells in

either acid alcohol or fresh sections are deeply stained by ruthenium red throughout their whole thickness, but the cuticle remains unstained. This accords with Mangin's results on cutinized membranes.

NERIUM OLEANDER.

Cross sections were cut through growing stems of various ages. The whole of the cambium wall is deeply and quite uniformly stained by ruthenium red either after or without previous treatment with acid alcohol. Similar results are attained by a few seconds' exposure to dilute methylene blue solution. The cambium walls are not stained by an exposure of three or four minutes to acid brown. The middle lamella of the xylem is deeply stained by methylene blue or ruthenium red, the other layers of the wall being colored very slightly if at all. The xylem walls are colored blue by methyl violet B. They are stained quite uniformly brown in a few minutes by acid brown. They are stained uniformly red in forty-five minutes by aniline-water-safranin. After thirty minutes' exposure to this safranin solution the xylem walls are colored bright red, the middle lamellae between the large tubes being decidedly darker than the thickening layers. After forty minutes' exposure to ponceau, the thickening layers of the xylem are stained somewhat, though not evenly, the middle lamella remaining uncolored.

Sections were exposed for twenty-four hours to neutral, acidulated, and alkaline solutions of orseille. The neutral and alkaline solutions produced the same coloration of the various tissues as the acidulated, but to a less degree. Therefore only the action of the acidulated solution will be described. The coloration of the xylem and medullary rays is a purplish-red, of all the other tissues affected, brown. The walls of the xylem have a general purplish tinge, but no distinctive staining is apparent in the different layers. The contents of the medullary ray cells are deeply colored, and the walls are stained similarly to those of the xylem. An exposure of two and one half hours to ponceau produces a reddish tinge in the xylem walls, and no further stain.

The contents of the medullary ray cells are stained by acid brown. Both the walls and the cell contents are deeply stained by methylene blue as well as by ruthenium red.

The results as to the tissues just described agree closely with those obtained for Pinus. The cambium walls and the middle lamellae of the xylem and medullary ray walls are of pectic nature; the remaining layers of the xylem and medullary ray walls are composed of a mixture of pectic and nitrogenous substances, the pectic constituents being perhaps predominant in the medullary rays; and there is evidence of the presence of a small proportion of cellulose in these thickening layers.

The bast fibers, when treated with ruthenium red, show a peculiar and most beautiful differential stain. It is most clearly brought out after treatment with acid alcohol, though it appears in sections not so treated. The middle lamella, which is relatively very thin, is deeply stained. It is sharply distinguished from the layer next within it on either side, which is colorless or very slightly stained. Next, passing toward the interior of the cell, comes a layer with a little color, then one a little more deeply stained, and so on, the depth of stain increasing until the last thickening layer, reaching almost to the center of the cell, is colored to about the same degree as the middle lamella. It should be noted that the stain shown in these walls is rather a purplish-red than the bright red commonly found in ruthenium red preparations. This behavior with ruthenium red is markedly different from that shown by Dippel (**2**) in his figure 139, in which all the wall of the bast fibers except the middle lamella is shown as unstained. On page 219, he mentions, as an instance of pectose-free cell walls, the bast fibers of *Nerium Oleander*. This is plainly an error.

The bast fibers are not stained at all by exposure for a few seconds to methylene blue. By methyl violet B (three or four minutes), the middle lamella of the bast and the other wall layers except those next the middle lamella are stained violet.

Acid brown has an effect exactly opposite to that of ruthenium red; that is, the middle lamella is not stained; the next layer is

quite deeply colored, the next is lighter, and the colors grow less and less intense until the innermost layers show no coloration at all. After a ten minutes' exposure to acid brown, sections were dehydrated, passed through clove oil, and mounted in Canada balsam, without affecting the staining, except that the color of the bast fibers was somewhat faded. The contents of the bast fibers are deeply colored by acid brown.

The action of orseille (acidulated solution, twenty-four hours exposure) is exactly like that of acid brown. The effect of aniline-water-safranin, on the other hand, is similar to that of ruthenium red.

Exposure to ponceau for various periods up to two and one half hours does not stain the bast.

By treatment for a few minutes with methyl violet B, the middle lamella of the bast and all the other layers except that next the middle lamella are stained violet.

It appears that in the bast, as generally elsewhere, the middle lamella is of pectic nature; that the first of the secondary layers is nearly or quite free from pectic substances, and is composed of a mixture of cellulose and nitrogenous substances, and that the subsequent layers gradually increase in pectic content at the expense of the cellulose and nitrogenous constituents, until the last layer is perhaps again purely pectic. But, from the purplish-red color given by ruthenium red and the indifference shown toward methylene blue, it may be inferred that the pectic compounds found in these walls are not exactly the same as those common to the middle lamellae of other tissues. These inner layers, like the pectic lining of the canals in the xylem of Pinus, show that the ability of the cell to secrete pectic acid is not limited to a single period of its development.

Acid alcohol sections treated for a few seconds with methylene blue show a deep blue color in the middle lamella of the collenchyma walls, much deeper than in the remaining portions of the wall, which, however, are also somewhat stained. The boundary between the middle lamella and the next adjoining layers is not so sharply defined as in the xylem and bast. At

the corners of the cells there is an angular, less deeply stained area enclosed by the middle lamella, just as in the fundamental parenchyma of Pteris.

The effect of ruthenium red on the collenchyma is similar to that of methylene blue. The collenchyma walls are not stained by acid brown (three or four minutes), but the cell contents are deeply colored. The contents are also stained brown by acidulated orseille solution, and the walls show a brownish tinge, but no distinctive stain for the different layers. The cell contents are stained by ponceau (forty minutes), but not the walls.

Acid alcohol sections treated for thirty minutes with aniline-water-safranin show a deeper stain in the middle lamella of the collenchyma than in the other wall layers.

The walls of the cork cells are not colored by methylene blue nor by acidulated orseille solution. Orseille also does not stain the cuticle.

In acid alcohol sections the middle lamella of the cork cells is stained more deeply by aniline-water-safranin (thirty minutes) than is the rest of the wall.

The pith cell walls take up ruthenium red freely, the middle lamella, which occupies the greater portion of the thickness of the wall, being deeply stained, especially at the corners. Sometimes the whole wall appears quite uniformly stained. The intercellular spaces are sometimes angular, but very often rounded, elliptical, or circular.

The contents of pith cells are stained by acid brown, the walls unstained. The action of orseille is similar. The failure of these two stains to affect the pith walls indicates that those walls are composed chiefly of pectic compounds, probably in purest form in the middle lamella.

ROSA SP.

In general, the results were the same as for Nerium. Cross sections were treated with acid alcohol and stained with ruthenium red. The cambium walls stain quite uniformly

throughout. The xylem shows a distinctive stain for the middle lamella, which is enlarged and especially deeply stained at the angles. The medullary ray walls and cell contents stain deeply.

The middle lamella and successive thickening layers of the bast fibers stain exactly as described for Nerium, including the purplish tinge. The stain of the middle lamella is deepest at the angles. The lining layer of the canals running from fiber to fiber is deeply stained where it traverses unstained or less deeply stained layers. This recalls the deeply stained layer of the pits of pine tracheids, and, with the deep stain of the last deposited wall layer in these tissues of the rose, shows these cells to possess the power very late in their history of depositing pectic wall material.

The pith cells show a very distinctive stain for the middle lamella.

In the collenchyma, the walls are stained throughout, the middle lamella much more deeply than the other layers, but the boundary between more and less deeply stained portions is not so clearly defined as in the xylem. The epidermal walls are stained about the same as the collenchyma. The cuticle is unstained.

Cross sections of a slightly older stem than that just described were stained with ruthenium red, then exposed for a few seconds to methylene blue. The xylem walls are stained purple by the combination, the middle lamella most deeply. The bast fibers take up both stains, the relative depth of stain of the various layers being about the same as with the red alone. The epidermal walls take up the blue more freely than the red. The collenchyma cells remain red.

Cross sections of a very young growing shoot were treated with acid alcohol and stained with ruthenium red. The cambium walls stained deeply throughout. Very thin tangential red lines are visible, representing the earliest deposit of wall material. Such very thin tangential walls are what one would expect to find in material gathered in the season of active cambial growth.

Young xylem elements have the walls entirely stained,

showing a long-continued period of pectic deposition. In older fibers a middle lamella is deeply stained, the remaining layers very slightly or not at all.

The bast fibers are stained throughout; no thickening layers are yet distinguishable. The middle lamella is already differentiated in the collenchyma, the other layers remaining uncolored. The middle lamella is especially deeply stained in the corners, and angular intercellular spaces are in some cases inclosed by it.

Similar sections not treated with acid alcohol yield similar results.

TILIA AMERICANA.

Cross sections through rather young growing shoots were treated with acid alcohol and ruthenium red.

The cambium walls stain deeply, in the youngest cells throughout their entire thickness. The radial walls are more deeply stained than the tangential, the corners especially deeply. Passing from the youngest cambium cells toward the bast or toward the xylem, the beginnings of the secondary thickening may be seen in a very thin unstained or only slightly stained layer of the wall within the stained portion. This is similar to the behavior of the cambium walls of pine, except that in Tilia, as generally, there is no evidence of a *Zwischensubstanz*, which, so far as I have observed, is found only in the pine cambium walls. The continuation of the deeply stained layer can be traced in the middle lamella of the much thickened xylem and bast elements.

The middle lamella of the xylem stains deeply, especially at the angles, where it is enlarged. The rest of the wall is slightly tinged with red.

The contents of the medullary ray cells are stained. Their walls are more uniformly stained than those of the xylem elements, but the middle lamella is still plainly distinguishable.

The bast has a deeply stained middle lamella, the other layers of the very much thickened wall being unstained or only slightly stained. In this respect, as in most others, the staining reactions of Tilia resemble those of pine.

The walls of the parenchyma cells between the bast bundles show a stain throughout, the middle lamella appearing thin and deeply stained, and somewhat enlarged at the angles.

In the collenchyma the whole wall and the cell contents are stained. Here, too, the middle lamella is thin and deeply stained, often including at the angles a less deeply stained area.

The walls of the cork cells stain quite uniformly throughout, but not very deeply. The color is purplish, different from the bright red typical of the middle lamella in other tissues. No middle lamella is here distinguishable. If anything, the innermost wall layer, next the lumen, is most deeply stained. Here, as in some of the tissues of Pteris, there is evidence that the middle lamella has undergone a chemical change, some or all of its pectic content having been replaced by other substances. The cuticle is unstained.

ILEX OPACA.

The tissues of the holly resemble those of the pine in their reactions to ruthenium red, the only stain I have used upon them.

In cross sections, either with or without previous treatment with acid-alcohol, the cambium walls are stained throughout by ruthenium red, and their connection may be traced with the middle lamellae of the xylem and bast.

The middle lamella of the xylem is thickened at the corners and stains deeply; the rest of the wall stains but slightly.

The middle lamella of the pith, which takes a deeper stain than the rest of the wall, is thickest at the angles, where it frequently encloses intercellular spaces. These spaces are sometimes triangular or quadrangular, but usually with more or less rounded angles, and often of an elliptical or circular form.

The middle lamella of the bast stains deeply, the rest of the wall slightly. The same is true in the collenchyma walls, where frequent angular intercellular spaces occur. The walls of the cork cells show no stain.

DISCUSSION.

In almost every tissue studied in which there was evidence of the deposition of wall layers at different periods, the staining reactions of the middle lamella showed it to differ in chemical composition from the adjoining layers; my results confirm Mangin's in every respect upon this point. Its distinctive character was brought out most clearly by ruthenium red. The reasons for concluding that, in general, this peculiarly staining layer is composed of pectic acid and its derivatives, have already been given at length. It does not follow, however, that its chemical composition is unchangeable. On the contrary, it is clear that, during cell growth and development, changes occur in the chemical constitution as well as in the form and mass of the middle lamella.

In pointing out the existence of a *Zwischensubstanz* between the radial walls of the cambial cells, and in distinguishing this layer from the "intercellular substance," either as characterized by Mangin or by himself, Dippel has contributed essentially to our clear conception of the conditions in the cambium of the pine; but his notion that the *Zwischensubstanz* is derived from the walls of the cambium mother cells has little evidence in its favor. I have never seen in this mass the fragments of these walls of which he speaks (**2**, p. 575). Besides, were Dippel's view correct, the tangential walls would be expected to contain at least as much of this substance as the radial walls; but, in fact, nothing of the sort is to be seen between the tangential walls, which, however, as shown in Dippel's own figures, are no thicker than are the radial walls without the *Zwischensubstanz*. On this point of the origin of the *Zwischensubstanz* Dippel is not clear, for he distinctly says (p. 575) that it is derived from the degenerated cambium mother cell walls, and again, on the same page, he speaks of the latter as going to make up the intercellular substance. It seems to me more probable that this *Zwischensubstanz* represents pectic acid which has exuded through the cambium cell walls into an intercellular cleft formed by the splitting of the radial walls, and that it is analogous to the lax substance

sometimes found at the angles of cells before the appearance of empty intercellular spaces. Its ultimate fate is doubtless to be absorbed into the adjacent cell walls, so that it does not appear as a visible layer in the middle lamella of mature tissues except in the case of the areas represented by the red-staining bars of the pine tracheids (see Dippel's figure 397), and except also, perhaps, on the surface of intercellular spaces. The intercellular spaces of the pine wood, then, are to be interpreted as remnants of the radial clefts between the cambium cells, rather than as newly formed in the adult tissue. But this does not preclude the probability of changes in the form and extent of the spaces due to the rounding up of the adjacent cells. It would be interesting to know whether this *Zwischensubstanz* is of wide occurrence. I have not observed it elsewhere than in the pine, and Mangin does not specifically note its existence.

A fact which is important as showing the plastic nature of the middle lamella at an early period of its history is its variation in thickness in different portions of older walls. This is shown commonly by an enlargement at the angles and a decrease in proportional thickness at the sides of the cells. Such variation is in marked contrast in the spermatophytes studied to the practical uniformity in thickness of the completely stained cambium walls. These are continuous with the middle lamella of the differentiated tissues, and, were it not for this change in form, would appear identical with the middle lamella. In some of the tissues examined, the substance of the middle lamella appears more dense at the enlarged angles, and seems to take up the characteristic stains more freely at those places. This appearance, however, may be accounted for by the optical effects produced by the greater thickness at the angles. But in other cases, as in the fundamental parenchyma of Pteris and the collenchyma of Nerium, these enlarged angles enclose a less deeply staining substance, or are becoming empty. These may be considered as stages in the development of the intercellular spaces, which are located, like the areas of less deeply stained substance just mentioned, at the angles formed by the junction

of three or four cells, are enclosed within the apparently split middle lamella, and are commonly angular in form.

Since there is no trace, except in the pine, of a *Zwischensubstanz* whose absorption would leave intercellular spaces, such spaces in the tissues of other plants can be accounted for only by the rounding up and drawing apart at their corners of adjoining cells. This induces a splitting of the middle lamella. The cleft so formed may be temporarily filled by a lax, fluid or semi-fluid substance, a solution, perhaps, of pectic acid or one of its transformation products, but this in time is absorbed into the walls, leaving an empty space. The surfaces of the wall exposed to the so-formed intercellular space may become further modified chemically, and even so softened as to flow enough to form a rounded instead of an angular space; but there is no evidence that such chemical changes ever occur except where the wall is exposed in an intercellular space, and it is more likely that the plasticity here displayed by the pectic acid has characterized it from the time of its deposition, but has not been shown so plainly because of its confinement within comparatively rigid limits.

On the basis of the later investigations respecting the relation of the cell wall to the cell plate, we have seen that the middle layer appearing after the splitting of the cell plate is to be considered as formed by deposition from the split halves of the original plate; the middle lamella of mature tissues would include, then, in addition to possible later deposits, both the layers deposited on the inner surfaces of the daughter plasma membranes. This being the case, we might expect to find in the history of the development of the middle lamella evidences that it consists of two layers. This is just what is found in the case of the intercellular spaces, which are very evidently caused by a split through the center of the middle lamella. If the middle lamella were not of a double nature, we should hardly expect it always to split through the middle, but, in view of its marked difference from the adjoining layers, we should expect that sometimes the whole layer would be pulled to one side or the

other as the cells round up and draw apart at their corners. As noted above, in sections split in cutting, there were also frequent cases where the middle lamella split rather than tore away from the other layers. The splitting in all these cases seems to indicate a weakness of cohesion in the plane between the two layers first deposited by the plasma membranes; and confirmation is thus given of the view advanced by Strasburger and confirmed by Timberlake that the cell plate splits before the new cell wall is laid down, the latter thus having a double nature from the start. We should not overlook the possibility that the very first layer which appears between the daughter plasma membranes is so thin as not to form a noticeable fraction of the thickness of the mature lamella; and that the splitting at a later stage in the middle lamella's history is due to a decomposition of this first thin layer, or that the split is really on one side or the other of it. I have, however, seen no evidence in support of either of these hypotheses.

It cannot be supposed that the middle lamella consists only of the material first deposited from the young plasma membranes. Its thickness in adult cells, and its varying thickness in different tissues of the same plant, *e. g.*, the large vessels and the sclerenchyma of Pteris, at once negative such an assumption. The older view, that it was the cell plate directly metamorphosed into cell wall material, is just as effectively negatived by the same considerations, which should have had more weight with the earlier observers. The middle lamella is rarely difficult of differentiation in adult thick-walled tissues simply by its greater density and less apparent stratification. Moreover, its growth in thickness can often be traced, for it stains continuously from the youngest wall (the thin tangential red lines in the cambium of the rose) to the middle lamella of mature tissues. We must conclude that the middle lamella consists of the layers first deposited by the plasma membranes *plus* a certain amount of material subsequently deposited in contact with these layers, which is generally rich in pectic compounds as compared with still later deposited strata. The middle lamella may vary

considerably in thickness in different tissues, according as a greater or less amount of material rich in pectic substance has been deposited in the cell. That it is not the only part of the cell wall, however, that contains pectic compounds is shown from the taking up by the other layers of the wall, though less freely than by the middle lamella, of pectic acid stains; it is shown also in the pectic layers lining the pits of the pine and the canals in the bast fibers of the rose, and in the innermost thickening layers of the bast of Nerium and rose.

Although the middle lamella usually retains its pectic nature, it undergoes a change by which, in the course of cell development, it loses the power of adapting itself to the varying form of the adjoining cells and becomes fixed and inflexible. The evidence seems satisfactory that this change, as Mangin suggests, is one from pectic acid to insoluble pectates, chiefly the calcium salt. Such a change is indicated by a less deep staining in older tissues, unless they are first treated with acid alcohol. A further transformation appears to take place in the cork cells of Tilia, by which the power of distinctive staining is entirely lost, and the middle lamella cannot be distinguished from the other layers of the suberized wall. The pectic acid here seems to have been partly replaced by suberin. The coloration of the cork walls is purplish, indicating a possible similarity in chemical nature to the later layers of the bast elements in Nerium and rose. In the sclerenchyma and stone cells of Pteris, also, though a middle lamella is plainly present, its staining reactions do not distinguish it from the rest of the wall; yet it can be traced as a continuation of the thin, characteristically-staining walls of the fundamental parenchyma. It is possible that the middle lamella in this case was from the start non-pectic; but it seems more probable that, as secondary thickening proceeded, the pectic acid was replaced or masked by material similar to that deposited to form the other layers. Further light could be thrown upon this question by tracing the development of the wall from the meristem to the mature tissues. The difference in depth of stain between radial and tangential walls in Tilia may indicate that the tangential

walls in this case are not composed of pure pectic acid, or that it exists in them in a somewhat different form. Such a difference in chemical nature might easily occur, since the tangential walls are being laid down anew, while the radial walls are older, and at most are simply added to as the cells increase in number and size. There is no evidence of the existence here of a mass corresponding to Dippel's *Zwischensubstanz*.

The power of the cell to secrete cell wall materials of very different chemical composition at different periods in its history is much greater even than is indicated by Mangin's work. That the compounds stained by ruthenium red are not derived from the decomposition of a previously deposited cellulose wall is proved by the complete staining of the young cambium wall. Still, that there is a possibility of a closer relation than Mangin supposes between cellulose and other cell wall materials on the one hand, and pectic substances on the other, perhaps even involving transformations of material from one class into the other, is to be inferred from the action of ruthenium red upon the walls of the cells surrounding the resin pores in pine. But that the power of secreting pectic compounds is not limited to a single period in the history of the cell is shown by the changes already discussed in the mass of the middle lamella. Since the very youngest and thinnest cambium walls are stained by ruthenium red, and at a later stage the thicker walls are still completely stained, the power of depositing pectic acid must last for a considerable time. That it continues in some cases even beyond the cambium stage is indicated by the variations in the thickness of the middle lamella in older tissues. Pectic substances, though perhaps not in the form of pectic acid, are also secreted at later stages in cell wall development, usually in combination with other materials, as cellulose, callose, and nitrogenous substances. In most of the thickened walls studied, evidence of the presence of pectic substances was found in the secondary thickening layers. The general rule, that pectic compounds are deposited in the cell wall early in the life of the cell, and that layers deposited later are predominantly non-pectic, is seen to have exceptions

in the case of the innermost strata of the bast fibers of Nerium and rose, and in the lining of the canals of the rose and of the pits of the pine. In these cases we have the deposition first of a pectic layer, then of non-pectic or mixed layers, and then again of a pectic layer. The purplish tinge imparted to the bast thickening layers just mentioned by ruthenium red indicates that these layers consist of some pectic substance or substances, other than pectic acid, at least in the form in which it exists in the cambium walls and the middle lamellae of other tissues.

Whether the period during which the cell generally deposits pectic acid, and thus forms the middle lamella, marks any special stage in its development which can be sharply distinguished from subsequent periods in its history is not certain. The evidence at present available seems to indicate that the pectic layer continues to increase in thickness about as long as the cambial cell is increasing in size. It is possible that the attainment by the cell of its adult size marks the limit of the growth of the middle lamella. In this case we might say that pectic acid is deposited so long as the metabolic processes of the cell result in a *plus* which is expressed in cell growth, but that later, when a metabolic equilibrium has been established, or when the excess of food is stored instead of being used for growth, or when the protoplast degenerates, a predominance of other cell-wall materials is deposited. The evidence for such a view, however, is far from complete. It must be remembered, too, that in the cambium cells the distinction between middle lamella and inner non-pectic layers is very early present, and that the middle lamella continues to increase in thickness after it is separated from the protoplast by the non-pectic strata deposited later. The middle lamella is not to be considered as consisting merely of the wall layers early deposited by the protoplast, without undergoing any later modification. It is quite possible that the pectic acid which characterizes it may in part be secondarily formed or deposited by infiltration through non-pectic layers which separate it from the protoplast. Some such hypothesis as this is necessary to account for its increase in

thickness during the later stages of cambial growth. Of interest upon this point is the fact noted by Mangin that, although in the meristem of some of the plant tissues studied by him no middle pectic layer could be detected by staining, yet, upon a treatment known in other cases to dissolve pectic acid, the cells are dissociated. My study of very young rose shoots, though not carried back far enough to dispute Mangin's results, yet shows that, at a point where the tissues have become very slightly differentiated from the meristem, the cambium walls show strongly by their affinity for ruthenium red their pectic nature.

CONCLUSIONS.

The facts that have been cited seem to me to show conclusively that the middle lamella is not merely the partition wall first laid down, either as a single or a double layer, by the plasma membranes. Nor is it, on the other hand, merely an intercellular substance of cement, a means for binding the cells together, as Mangin holds. Individual cells are not separated from one another, either in their formation or their later development, and no reason appears why an intercellular cement should be secreted. There is, however, abundant reason why, in woody tissues especially, there should be during cell growth a plastic region in the cell wall, which should be in a measure adaptable to the changing size and form of the protoplast itself, and to the firm, resistent layers whose form must correspond to that of the protoplast at the time of their deposition. The middle lamella is, therefore, a wall layer with a complicated history, undergoing after its first appearance changes in form, increase, and probably at times decrease in mass, and changes in chemical composition; its history, too, is not identical in different tissues. There can be no doubt that, in the higher plants studied, the young cambium walls are included in, and form the basis of, the middle lamella of the older tissues. These may at first include, at least in the pine, an amorphous *Zwischensubstanz*, which occupies a cleft formed by the splitting of the young radial walls. Quite early in the history of the cambium cells, a non-pectic layer

appears within the first-formed pectic stratum; but the latter, now properly a middle lamella, continues to increase in thickness even after the appearance of the non-pectic layer. The substance of the middle lamella, now, is not rigid, but is more or less plastic, or even, as Dippel believes, soluble under certain conditions of development. Its solubility may lead at times to its partial absorption by the rest of the wall. More often, probably, its mass is increased by further secretion of pectic material from the adjoining cells into the spaces formed by their rounding up. The plastic nature of the whole layer allows the modification of its shape, also, as the cells round up and as more resistent layers are deposited against it, leading to its being massed at the corners and pressed out to a thinner layer at the sides. The pressure may be in certain cases so great at the corners as to increase the density at those places; but in general, as the cells round up and draw away from the corners, the pressure there is relaxed, and, if new material is not deposited, the substance becomes less dense, perhaps swelling by the absorption of water. Or it may be that already the middle lamella has split, and the cleft has been filled by the exudation into it of a pectic fluid, which is later to be reabsorbed. At any rate, sooner or later the tension incident to the rounding-up process brings about a split in the middle lamella, and an intercellular space is formed. In case a *Zwischensubstanz* was present, a splitting at this time is unnecessary, and the intercellular space results from the absorption of this substance. If the substance of the middle lamella is sufficiently rigid, the intercellular space remains angular; but if the material still retains some plasticity, or if it undergoes a chemical change upon its exposure at the newly formed surface, the angles may be rounded, or may disappear entirely, leaving a circular or elliptical space. The chemical changes effected in the middle lamella after the cell has attained its final form consist in the conversion of pectic acid into insoluble pectates, or even, as in the cork cells of Tilia, its replacement by entirely different substances.

It seems to me plain that the term "middle lamella,"

indicating merely the position of the layer in question, is preferable to either "cement" or "intercellular substance." The latter term, arising from a totally false conception as to the origin of cells, should certainly be dropped. There is also no evidence that the cell wall layers which, taken together, form the middle lamella have any significance as a cement for binding the cells together, and so it is inappropriate to apply to them a name suggesting any such function.

The fact of the origin of the middle lamella as a double layer deposited on the surfaces of two plasma membranes, and the fact that in the formation of intercellular spaces it always splits in a median plane, must always be borne in mind. This constant plane of cleavage may be called the primary cleavage plane, or, perhaps, the primary cleft when it is spoken of with reference to the space between the daughter plasma membranes in which the cell wall material is deposited.

I would distinguish cambial walls as the walls of cells yet capable of division, and primary cell walls as those layers added during the growth of the cambium cell into a full-sized wood or bast element. Secondary thickening would include the strata deposited in the subsequent history of such cells. This secondary thickening may be subdivided further where evidence exists that its deposition has been interrupted and subsequently resumed; we should then have tertiary thickening, and so on.

For the adult condition of thickened cell walls, the following terminology might be proposed as most exact from the standpoint of our present knowledge:

The boundary plane between adjacent cells, commonly invisible except where indicated by intercellular spaces, would be, following the suggestion already made, the primary cleavage plane. Including this on both sides, we should have the apparently homogeneous middle lamella, including the cambial walls and more or less of the primary cell walls, according to the proportional pectic acid content of the latter. Then would come those layers of the primary wall, if there be such, which are predominantly non-pectic; and lastly the layers of secondary and

perhaps tertiary thickening formed in the metamorphosis of the adult cell into its permanent condition as the element of a tissue.

The investigations here discussed were undertaken at the suggestion of Professor R. A. Harper, and have been carried on under his direction. All the results accomplished are due in the highest degree to his suggestions and assistance.

UNIVERSITY OF WISCONSIN.

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